Determination of reference intervals and comparison of venous blood gas parameters using standard and non-standard collection methods in 24 cats

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Abstract: OBJECTIVES: The aim of this study was to determine in-house reference intervals (RIs) for venous blood analysis with the RAPIDPoint 500 blood gas analyser using blood gas syringes (BGSs) and to determine whether immediate analysis of venous blood collected into lithium heparin (LH) tubes can replace anaerobic blood sampling into BGSs. METHODS: Venous blood was collected from 24 healthy cats and directly transferred into a BGS and a LH tube. The BGS was immediately analysed on the RAPIDPoint 500 followed by the LH tube. The BGSs and LH tubes were compared using paired t-test or Wilcoxon matched-pairs signed-rank test, Bland-Altman and Passing-Bablok analysis. To assess clinical relevance, bias or percentage bias between BGSs and LH tubes was compared with the allowable total error (TEa) recommended for the respective parameter. RESULTS: Based on the values obtained from the BGSs, RIs were calculated for the evaluated parameters, including blood gases, electrolytes, glucose and lactate. Values derived from LH tubes showed no significant difference for standard bicarbonate, whole blood base excess, haematocrit, total haemoglobin, sodium, potassium, chloride, glucose and lactate, while pH, partial pressure of carbon dioxide and oxygen, actual bicarbonate, extracellular base excess, ionised calcium and anion gap were significantly different to the samples collected in BGSs ($P < 0.05$). Furthermore, pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, ionised calcium and anion gap exceeded the recommended TEa. CONCLUSIONS AND RELEVANCE: Assessment of actual and standard bicarbonate, whole blood base excess, haematocrit, total haemoglobin, sodium, potassium, chloride, glucose and lactate can be made based on blood collected in LH tubes and analysed within 5 mins. For pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, anion gap and ionised calcium the clinically relevant alterations have to be considered if analysed in LH tubes

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Original Article

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Determination of reference intervals and comparison of venous blood gas parameters using standard and non-standard collection method in 24 cats

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Abstract

Objective

The aim of this study was to determine in-house reference intervals for venous blood analysis with the RAPIDPoint 500 blood gas analyzer using blood gas syringes (BGS) and to determine whether immediate analysis of venous blood collected into lithium heparin (LH) tubes can replace anaerobic blood sampling into BGS.

Methods

Venous blood was collected from 24 healthy cats and directly transferred into a BGS and a LH tube. The BGS was immediately analyzed on the RAPIDPoint 500 followed by the LH tube. The BGS and LH tubes were compared using paired t-test or Wilcoxon matched-pairs signed-rank test, Bland-Altman and Passing-Bablok analysis. To assess clinical relevance, bias or % bias between BGS and LH tubes was compared with the allowable total error (TEa) recommended for the respective parameter.

Results

Based on the values obtained from the BGS, reference intervals were calculated for the evaluated parameters including blood gases, electrolytes, glucose and lactate. Values derived from LH tubes showed no significant difference for standard bicarbonate, whole blood base excess, hematocrit, total hemoglobin, sodium, potassium, chloride, glucose and lactate while pH, partial pressure of carbon dioxide and oxygen, actual bicarbonate, extracellular base excess, ionized calcium and anion gap were significantly ($p < 0.05$) different to the samples collected in BGS. Furthermore pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, ionized calcium and anion gap exceeded the recommended TEa.

Conclusions and relevance

Assessment of actual and standard bicarbonate, whole blood base excess, hematocrit, total hemoglobin, sodium, potassium, chloride, glucose and lactate can be made based on blood collected in LH tubes and analyzed within 5 minutes. For pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, anion gap and ionized calcium the clinically relevant alterations have to be considered if analyzed in LH tubes.

Keywords

feline, venous blood gas, acid base, ionized calcium, emergency, critical care

Introduction

In recent years the use of blood gas analyzers for rapid determination of oxygenation, ventilation, acid-base and electrolyte disorders in blood has become a standard in emergency rooms and critical care units of veterinary hospitals. The RAPIDPoint 500 (RP500, Siemens Healthcare) allows point of care assessment of blood gases, acid base status, co-oxymetry, electrolytes, glucose and lactate within 60 seconds from a single whole blood sample.

While interpretation of oxygenation requires analysis of an arterial blood specimen, several studies in humans concluded that measurements from venous blood accurately reflect the acid-base status of the patient.[1,2,3] In cats and dogs the venous pH is significantly lower compared to arterial pH while venous partial pressure of carbon dioxide ($PvCO_2$) is significantly higher than arterial partial pressure of carbon dioxide ($PaCO_2$), subsequently leading to significantly higher bicarbonate (HCO_3^-) concentrations but no significant changes in base excess (BE).[4,5] For the correct assessment of venous blood gases reverence intervals (RI) for venous blood have to be determined.

Hematocrit, total hemoglobin, electrolytes, glucose and lactate are typically measured in venous blood. A venous sample is easier to obtain and facilitates evaluation of acid-base status, electrolyte disorders and glucose and lactate concentration in the emergency situation.

Manufacturers of blood gas analyzers recommend using specific blood gas syringes (BGS) to obtain reliable results. The smallest syringes available have a volume of 1 ml. Cats are small and have a lower relative blood volume than dogs. Small amounts of blood withdrawal are therefore desirable. Measurement of venous blood gas parameters using multi-purpose lithium heparin (LH) tubes which subsequently can be used for analysis of other blood parameters decreases the amount of required blood. It has been stated that canine venous blood samples collected into LH tubes didn't show significant changes in $PvCO_2$, pH, HCO_3^- and BE on eight different timepoints within 30 minutes except for pH at two minutes after sample collection compared to native blood analyzed directly after collection.[6]

The aim of this study was to determine in-house reference intervals for venous blood analysis with the RP500 blood gas analyzer using BGS and to determine whether immediate analysis of venous blood collected into LH tubes can replace anaerobic blood sampling into BGS in cats.

The null hypothesis was that venous blood samples collected in BGS and their corresponding venous blood samples collected in LH tubes deliver the same results for the evaluated parameters when analyzed within 5 minutes on the same blood gas analyzer.

Materials and Methods

Animals and Procedures

The study was approved by the Swiss federal ethics committee on animal research of the Canton of Zurich. Cats older than one year were recruited between April 2013 and January 2014. They were considered healthy based on history, physical examination, hematology and serum biochemistry (parameters specified in Table S1). Informed owner consent was obtained for all procedures.

Blood was sampled from the jugular vein with the cats restrained in sternal recumbency. The head was flexed dorsally and the forelimbs extended downwards over the edge of a table. If cats didn't tolerate this position without struggling they were positioned in lateral recumbency and blood was collected from the medial saphenous or the cephalic vein. The cats were not sedated. After disinfection of the skin, blood was aspirated into a 10 ml syringe (Omnifix, B Braun Medical AG) using a 22 G hypodermic cannula. Subsequently the blood was filled into the following containers: 0.6 ml blood into a 1 ml blood gas syringe (BD Preset 1 ml, 30 I.U. calcium balanced lithium heparin; Becton, Dickinson and Company), 1.3 ml blood into a LH tube (35 I.U. lithium heparin per ml blood; Sarstedt), 1.3 ml blood into a potassium-EDTA tube (Sarstedt) and 1 ml blood into a serum tube. Air bubbles in the BGS were immediately expelled and the syringe closed with a rubber cap. The LH tube was closed with a plastic screw cap. The BGS and corresponding LH tube were then analyzed immediately with the RP500 (Siemens Healthcare; analytical performance in the supplementary material). This analyzer uses ion-selective electrodes for analysis of pH, Na, K, iCa and Cl, modified potentiometry for PCO_2 (Severinghaus electrode) and amperometry for PO_2 (Clark electrode), glucose and lactate (enzyme electrodes). Quality control is performed three times daily using an automatic quality control cassette. Parameters not passing the analytical performance criteria (Table S2) are transiently deactivated. The BGS was analyzed first, followed by the LH tube. Blood gas syringes were connected to the sample receipt of the analyzer. For the LH tubes the screw cap was opened immediately prior to analysis and 0.2 ml of blood (amount required for the analysis) was aspirated into an uncoated 1 ml PVC syringe (Omnifix, B Braun Medical AG) and the syringe was then connected to the analyzer. The potassium-EDTA tube and the serum tube were sent to the in-house laboratory for a haematology and serum biochemical profile. Sixteen different parameters provided by the blood gas analyzer were analyzed including pH, venous partial pressure of carbon dioxide ($PvCO_2$), venous partial pressure of oxygen (PvO_2), actual bicarbonate (HCO_3^-act), standard bicarbonate (HCO_3^-std), whole blood base excess (BE B), extracellular base excess (BE ecf), hematocrit (Hct), total hemoglobin (tHb), sodium (Na), potassium (K), ionized calcium (iCa), chloride (Cl), anion gap (AnGap), glucose and lactate.

Statistical analysis

Data was analyzed using Excel (Microsoft Corporation) and two statistical software packages (GraphPad Prism 6, GraphPad Software Inc. and Analyse-it, Analyse-it Software Ltd.). Shapiro-Wilk test was performed to confirm or reject normal distribution for every measured parameter. In-house reference intervals based on the immediately analyzed samples collected in the BGS were determined with the Reference Value Advisor add-in for Microsoft Excel.[7] In agreement with the ASVCP guidelines for the determination of reference intervals in veterinary species[8] a parametric method was used (recommended for sample sizes of 20 – 40 samples with normal distribution). For parameters not showing normal distribution, values were box-cox transformed to achieve normality. 90% Confidence intervals were calculated for upper and lower limits according to the guidelines.[8] Differences between results from BGS and LH tubes were analyzed using paired t-test (confirmed normality) or Wilcoxon matched-pairs signed-rank test (rejected normality). Bland-Altman[9] and Passing-Bablok[10] analysis were used to evaluate the agreement between the two methods. To assess clinical relevance the bias was compared with the allowable total error (TEa) of the respective parameter according to the American College of Veterinary Pathologists (ASVCP) guidelines for allowable total error.[11] For parameters not defined in these guidelines TEa values defined for human medicine were used. For parameters where TEa is expressed in percent, % bias was calculated using BGS as the gold standard (% bias = bias/mean of BGS). Level of significance was set at $P<0.05$.

Results

Animals

Twenty-five healthy cats were enrolled in the study. One cat had to be excluded since air hadn't been properly expelled from the BGS before capping. Age of the remaining 24 cats ranged between 1 and 15 years with a median of 4 years. Most cats (18) were domestic shorthair, the other breeds represented were Maine Coon (2), British Shorthair (2), Persian (1) and domestic longhair (1). Twelve cats were male neutered, one male entire and 11 female spayed.

Reference intervals (RI) were calculated from 24 cats. Two cats had to be excluded from the comparison between standard and non-standard collection method since the samples were analyzed in the wrong order.

Twenty-two cats were sampled on the jugular vein and two cats on the medial saphenous and the cephalic vein, respectively.

Reference intervals

Values for the parameters Na, iCa, Cl, glucose and lactate were not normally distributed while the other nine parameters showed normal distribution. Tukey test identified suspect data for Na, iCa and glucose (one sample each). These values are included in the RI since they originated from different individuals and no evidence for pre-analytical error or inclusion of unhealthy animals was detected. Hematocrit and tHb were only measured in 9 individuals, therefore no RI is reported. Values for iCa were available in 14 animals and only mean, median, SD, minimum and maximum are reported (Figure 1 and Table 1).

Comparison of blood gas syringes and lithium heparin tubes

Values derived from LH tubes showed no significant difference for the parameters HCO_3^- std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate while the parameters pH, $PvCO_2$, PvO_2, HCO_3^-act, BE ecf, iCa and AnGap were significantly different compared to the samples collected in BGS (Table 2).

Time between the two measurements ranged between 2 and 5 minutes with a mean of 2.7 minutes and a standard deviation of 0.8 minutes.

Analysis of the Bland-Altman plots revealed high bias and wide LOA for $PvCO_2$ and PvO_2. Smaller bias and narrower LOA were observed for pH, HCO3-act, BE ecf, iCa and AnGap. The nine parameters not showing significant difference (HCO_3^-std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate) all had small bias and narrow LOA (Table 2 and Figure 2).

Comparison of % bias with TEa as defined by the ASCVP guidelines showed that the parameters actual and standard bicarbonate, Na, K, Cl, glucose and lactate are within the proposed limits. All other parameters were compared to human recommendations. Bias or % bias of pH, $PvCO_2$ and iCa exceeded human TEa. The parameters PvO_2 (7/22), AnGap (5/22) and BE ecf (2/22) showed samples exceeding human TEa. One of the samples exceeded TEa for BE B but t-test showed no significant difference between BGS and LH tube for this parameter ($p = 0.5037$). The % bias of the two parameters hematocrit and total hemoglobin is within the human recommendations (Table 3).

Passing-Bablok regression revealed proportional error for pH, $PvCO_2$, PvO_2, Na, iCa and AnGap (Figure 3).

Discussion

Critical illness in cats may lead to abnormalities in oxygenation/ventilation, acid-base hemostasis, hematocrit, electrolytes, glucose or lactate, among others. Blood gas analyzers measuring these parameters allow immediate identification of potentially life-threatening abnormalities with a small blood sample. To our knowledge, this is the first

study reporting feline venous blood gas RI for the RAPIDPoint 500 and showing that samples collected in multipurpose LH tubes allow clinically accurate determination for some of the parameters.

Reference intervals depend on the specific method of measurement and need to be determined for each analyzer. Studies on canine and feline acid-base values showed that acid-base values in healthy cats differ from values measured in healthy dogs,[4,12] illustrating the importance to use species specific RI to interpret blood gas results. Only a few studies have investigated feline venous blood gas RI and they have been established on small numbers of five,[4] eight,[12] ten[13] and thirteen[14] animals respectively. Our samples were obtained by venipuncture of conscious and restrained cats while some of the previous studies on conscious cats used indwelling catheters for sampling.[4,13] Our results therefore include possible variations induced by stress as it might occur in a clinical situation. This led to a broader RI compared to the studies were blood was sampled from indwelling catheters. Apart from a considerably lower minimum level for $PvCO_2$ and a higher upper limit for pH our RI confirm the RI reported by Middleton et al based on 13 cats.[14] The discrepancy in pH and $PvCO_2$ might be a result of hyperventilation and consequently respiratory alkalosis due to stress during venipuncture or represent a higher biological variation due to the larger sample size in our study. The RI for PvO_2 is of minor clinical relevance as oxygenation is generally assessed in arterial blood.

For lactate there is an ongoing debate about the width of the reference interval in this species and possible influences of factors like stress,[15,16] sampling technique, sample handling and sample population.[17] The most recent study published 2015 by Tynan et al. on 47 cats determined a broader reference interval (0.67 – 5.44 mmol/l)[17] than previously suggested.[12,16] Our study supports the results of Tynan et al with a similar reference interval of 0.61 – 5.86 mmol/l for our 24 cats. Further studies to assess possible factors leading to this high variation of lactate concentration in healthy cats are required.

A limitation of our study is the number of cats involved. Generally, RI should be based on the largest number of samples possible. While in human medicine a minimum of 120 samples is required, the ASVCP guidelines for the determination of RI in veterinary medicine advises against calculating RI with a sample size below 20 and recommends a sample size $\geq$ 40 individuals.[8] Our sample size of 24 individuals is relatively small but allows reporting RI with a reasonable validity. Hematocrit and tHb was only available in 9 cats since the spectrophotometric unit of the analyzer was not available for some days of the study. This missing data also has an impact on HCO_3^-std and BE B since tHb is used for calculation of these parameters. If tHb isn't available, 15 g/dl is used as default value in the respective formulas. Since all cats showed tHb within RI (11.3 – 15.5 g/dl) in hematology performed in our in-house laboratory, this effect is negligible. A malfunction of the ion selective electrode for iCa reduced the number of values for this parameter to 14 with no influence on other parameters. Our reported minimum and maximum for iCa however permits an estimate of the physiological range of this parameter in healthy cats.

Not differentiating between samples from different venous sites conflicts with the principle of maximum standardization for the determination of RI, however only 2 of 24 samples were not aspirated from the jugular vein. Sampling from different venous sites reflects clinical practice and for venous acid-base status[4] and lactate concentration[16] it has been demonstrated that results are comparable between different venous sites in cats.

Of the 16 parameters analyzed in this study, HCO_3^-std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate showed no significant differences in measurements between sampling in BGS and LH tubes. Consequently, medical conditions mainly influencing these parameters are reliably diagnosed with blood collected in LH tubes. The parameters pH, $PvCO_2$, PvO_2, HCO_3^-act, BE ecf, iCa and AnGap demonstrated significant changes in the LH tubes.

The main differences between the two sample containers used in this study are the exposure to room air and the anticoagulant. While the BGS are completely filled with blood up to the cone and air is expelled before capping, the LH tubes are designed for 1.3 ml of blood but have a total volume of about 2 ml, leaving 0.7 ml of room air inside the tube after capping. These tubes have been chosen for the study since they are standardly used for collection of blood for analysis on our emergency biochemistry analyzer during emergency service times. It has been shown previously in humans that air bubbles left in a syringe lead to a significant decrease in $PvCO_2$ within three minutes and a significant rise in PvO_2 within only two minutes.[18] Exposure to room air allows diffusion of an adequate amount of O_2 from room air with a higher PO_2 to blood and of CO_2 from blood with a higher PCO_2 to room air to change these two parameters significantly.

Our study shows a significant increase of PvO_2 and pH combined with a significant decrease of $PvCO_2$, HCO_3^-act and BE ecf in the tubes. The loss of CO_2 to room air in the tube leads to a significant decrease in PvCO2 and subsequently to a significant increase in pH. Based on the formulae used for calculation of HCO_3^-act and BE ecf which contain pH and pCO_2 these two parameters are lower in the LH tubes. For the calculation of HCO_3^-std and BE B changes in pH and pCO_2 have less influence, therefore no significant difference was observed. Since BE ecf is typically preferred over BE B in acid-base analysis[19] this significant difference might be clinically relevant.

The study from 2004 by Rickey et al[6] comparing canine native venous blood analyzed directly after collection with samples stored in the same LH tubes as in our study did not find significant changes in $PvCO_2$,HCO_3^- and base excess on eight different timepoints within 30 minutes. Two minutes after sample collection pH was higher compared to native blood.[6] Comparing the two timepoints 2 minutes and 5 minutes after sample collection from that study with our samples, their samples showed the same tendencies though didn't reach significance except for the rise in pH after 2 minutes.

The observed rise of the anion gap in our study is a further consequence of the decreased HCO_3^-act value. Anion gap is calculated using the formula

$$Na + K - (Cl + HCO_3^-act)$$

and therefore a lower HCO_3^-act value leads to a higher calculated anion gap.

The significant change in iCa between the samples collected in the blood gas syringe and the samples in the LH tubes is most likely based on the type of anticoagulant used. Heparin complexes calcium and thereby reduces the amount of measurable ionized calcium. Commercial blood gas syringes use dry calcium balanced lithium heparin, which contains calcium to 'balance' the amount of iCa complexed by the heparin. Human studies support the use of calcium balanced heparin for iCa analysis.[20,21] A rise in pH also reduces concentration of iCa in blood. The observed decrease of iCa (0.07 mmol/l) in the tubes exceeds the effect expected by the rise in pH of 0.04 according to a human study[22] and was probably caused by the non-balanced heparin complexing part of the iCa.

Statistical significance does not necessarily implicate clinical relevance. A difference in measured values of a certain parameter is only clinically relevant if it leads to a different decision regarding further treatment of the patient. The threshold for relevance varies greatly between different parameters depending on how strictly physiologically regulated they are in vivo.[23] Considering this, the concept of allowable total error (TEa) has been introduced in laboratory quality control. The specific TEa for a parameter of interest is derived from biological variation or clinical decision threshold and can vary between different species, analyte concentrations, clinical use and type of laboratory.[11] In human medicine, hundreds of parameters have been assessed and TEa for each parameter has been defined. For veterinary medicine the ASVCP total allowable error guidelines for biochemistry[11] is the only source to date. The parameters measured in our study, which are mentioned in these guidelines (sodium, potassium, chloride, glucose lactate and bicarbonate) all show a % bias lower than the recommended TEa, suggesting that the significant difference in HCO_3^-act may not be clinically relevant. Since the bias for pH, $PvCO_2$ and iCa exceeded human recommendations and PvO_2 AnGap and BE ecf showed samples exceeding human TEa these differences presumably are clinically relevant in cats.

Using single instead of repeated testing may lead to over- or underestimation of the difference between the two methods for each sample pair but the calculated bias refers to the mean difference of all 24 samples and therefore provides an accurate assessment of the difference between the two containers. No internal validation to confirm the analytical performance stated by the manufacturer was performed for our RP500. However, the automated quality control performed three times daily confirmed accurate measurement within the predefined range.

Conclusion

Assessment of HCO_3^-act, HCO_3^-std, BE B, Hct, tHb, Na, K, CL, glucose and lactate can be made based on blood collected in LH tubes. For pH, $PvCO_2$, PvO_2, BE ecf, AnGap and iCa the clinically relevant alterations have to be considered if analyzed in LH tubes.

Conflicts of interest

The authors do not have any potential conflicts of interest to declare.

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Table 1: Reference intervals of feline venous blood gas parameters

Parameter	n	Unit	Mean	Median	SD	Min	Max	Reference Interval	lower 90% CI	upper 90% CI
pH	24		7.344	7.343	0.048	7.230	7.423	7.244 - 7.444	7.218 - 7.272	7.415 – 4.472
$PvCO_2$	24	mmHg	38.2	37.6	5.2	30.7	49.2	27.3 – 49.1	24.5 – 30.3	45.9 – 52.1
PvO_2	24	mmHg	45.1	45.7	5.3	34.5	54.0	33.9 – 56.3	31.0 – 37.0	53.1 – 59.4
HCO_3^-act	24	mmol/l	20.3	20.6	2.1	16.9	24.4	15.9 – 24.7	14.7 – 17.1	23.4 – 25.9
HCO_3^-std	24	mmol/l	20.0	19.9	1.7	16.8	24.0	16.4 – 23.6	15.5 – 17.4	22.6 – 24.6
BE B	24	mmol/l	-4.9	-5.0	2.2	-9.2	-0.3	-9.5 – -0.3	-10.6 - -8.2	-1.6 – 1.0
BE ecf	24	mmol/l	-5.5	-5.5	2.4	-9.5	-0.7	-10.4 – -0.5	-11.7 - -9.1	-1.9 – 0.9
Na*	24	mmol/l	153.9	153.9	1.6	149.8	155.7	150.5 – 157.2	149.4 – 152.1	156.4-157.8
K	24	mmol/l	3.88	3.91	0.36	3.19	4.76	3.11 – 4.64	2.92 – 3.32	4.42 – 4.85
iCa*	14	mmol/l	1.29	1.30	0.04	1.19	1.35	-	-	-
Cl*	24	mmol/l	118	119	2	113	121	113 – 123	112 – 115	122 – 124
AnGap	24	mmol/l	19.3	19.2	2.1	15.4	23.4	14.8 – 23.8	13.7 – 16.1	22.5 – 25.0
Glucose*	24	mmol/l	5.8	5.6	1.1	4.4	9.0	4.3 – 8.8	4.1 – 4.6	7.5 – 10.7

Lactate*	24	mmol/l	2.32	1.85	1.24	0.50	5.23	0.61 – 5.86	0.41 – 0.89	4.39 – 7.50

Table 1: n: number of samples, SD: standard deviation, Min: minimum value, Max: maximum value, CI: confidence interval. * Parameters not showing normal distribution. For iCa no reference interval was determined and only mean, median, sd, min and max are reported.

Table 2: Comparison of feline blood gas parameters determined by blood gas syringes and lithium heparin tubes

Parameter	n	Unit	Mean BGS	Mean LH tube	Bias	95% CI	Limits of agreement		P-Value
pH*	22	-	7.343	7.389	0.046	0.038 – 0.054	0.010	0.082	<0.0001
$PvCO_2$*	22	mmHg	38.3	32.0	-6.3	-7.8 – -4.8	-13.0	0.4	<0.0001
PvO_2*	22	mmHg	44.7	49.7	5.0	2.8 – 7.1	-4.6	14.5	<0.0001
HCO_3^- act*	22	mmol/l	20.3	18.8	-1.5	-1.9 – -0.9	-3.7	0.8	<0.0001
HCO_3^-std	22	mmol/l	20.0	20.0	0	-0.1 – 0.3	-0.9	1.0	0.4871
BE B	22	mmol/l	-4.9	-5.0	-0.1	-0.4 – 0.2	-1.5	1.3	0.5037
BE ecf*	22	mmol/l	-5.5	-6.2	-0.7	-1.1 – -0.3	-2.6	1.2	0.0025
Hct	7	%	43.6	43.4	-0.2	-0.5 – 0.2	-0.9	0.6	0.3559
tHb	7	g/l	14.8	14.8	0	-0.1 – 0.1	-0.2	0.2	0.7358
Na	22	mmol/l	153.8	153.5	-0.3	-0.9 – 0.3	-2.9	2.3	0.2122
K	22	mmol/l	3.91	3.87	-0.03	-0.08 – 0.01	-0.21	0.14	0.0912
iCa*	13	mmol/l	1.29	1.22	-0.07	-0.10 – -0.03	-0.19	0.06	0.0015

Cl	22	mmol/l	118	118	0	-0.3 – 0.6	-1.9	2.2	0.6606
AnGap*	22	mmol/l	19.3	20.3	1.0	0.2 – 1.8	-2.6	4.5	0.0197
Glucose	22	mmol/l	5.8	5.8	0	-0.2 – 0.1	-0.7	0.6	0.5080
Lactate	22	mmol/l	2.4	2.5	0.1	-0.01 – 0.12	-0.2	0.3	0.0751

Table 2: n: number of samples, BGS: Blood gas syringe, LH tube: multi-purpose lithium heparin tube, CI: confidence interval of the bias. P-Values correspond to difference between blood gas syringes and lithium heparin tubes. * Parameters showing significant ($p < 0.05$) difference between blood gas syringes and lithium heparin tubes.

Table 3: Bias or % bias compared to recommended allowable total error (TEa)

Parameter	Bias/% bias	Range of bias	TEa	Source	Samples exceeding TEa
pH*	0.046	0.007 – 0.076	0.04	CLIA	14
$PvCO_2$*	-6.3 mmHg -16.4%	-13.8 – 3.1 mmHg -28.7 – 9.3%	5 mmHg or 8%	CLIA	16
PvO_2*	5.0 mmHg 11.2%	0.5 – 18.1 mmHg 1.1 – 37.4%	5 mmHg or 5%	RCPA	7
HCO_3^-act	-7.4%	-16.5 – 11.1%	20%	ASVCP	0
HCO_3^-std	0%	-3.3 – 7.2%	20%	ASVCP	0
BE B*	-2.0%	-133.3 – 22.7%	85%	BV	1
BE ecf*	12.7%	-171.4 - 24.1%	85%	BV	2
Hct	-0.5%	-2.3 – 0%	6%	CLIA	0
tHb	0%	-1.4 – 0.8%	7%	CLIA	0
Na	- 0.2 %	- 1.9 – 1.8%	5%	ASVCP	0
K	-0.9%	-4.8 – 3.5%	5%	ASVCP	0

iCa*	-5.1%	-16.0 – 1.6%	2%	BV	10
Cl	0%	-1.7 – 1.7%	5%	ASVCP	0
AnGap*	5.2%	-7.4 – 33.8%	11.3%	BV	5
Glucose	0%	-9.5 – 15.6%	20%	ASVCP	0
Lactate	4.2%	-9.8 – 28.0%	40%	ASVCP	0

Table 3: ASVCP: American Society for Veterinary Clinical Pathology, CLIA: Clinical Laboratory Improvement Amendments Proficiency Testing Limits (1988), RCPA: Royal College of Pathologists of Australasia and the Australasian Clinical Biochemist association Quality Assurance Program, BV: Spanish Society of Clinical Chemistry and Molecular Pathology table of Desirable Quality Specifications based on Biological Variation (Update 2004). * Parameters containing samples exceeding recommended TEa

Figure 1: Histograms showing distribution of analyzed parameters in 24 cats

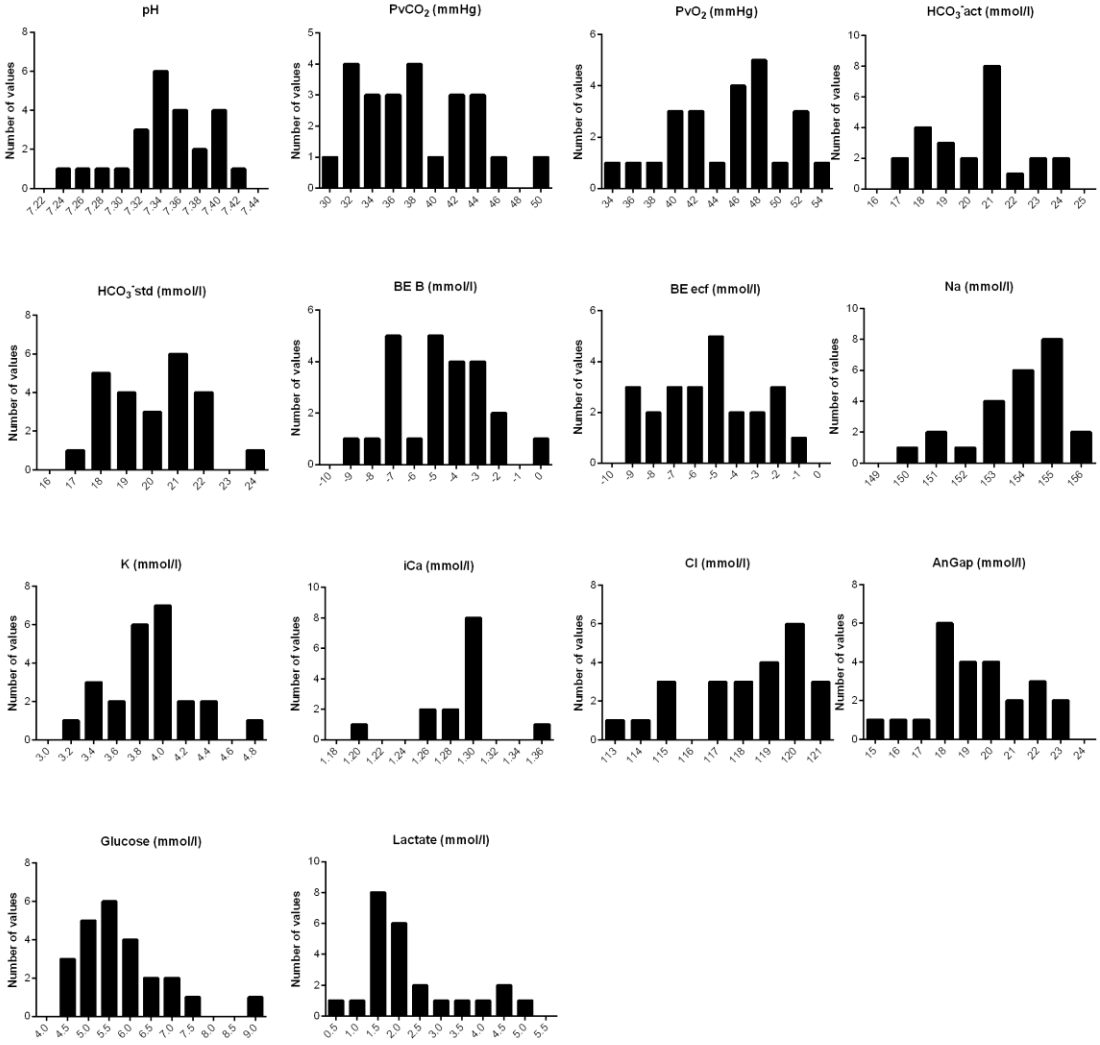

Figure 2: Bland and Altman plots comparing blood gas syringes and lithium heparin tubes

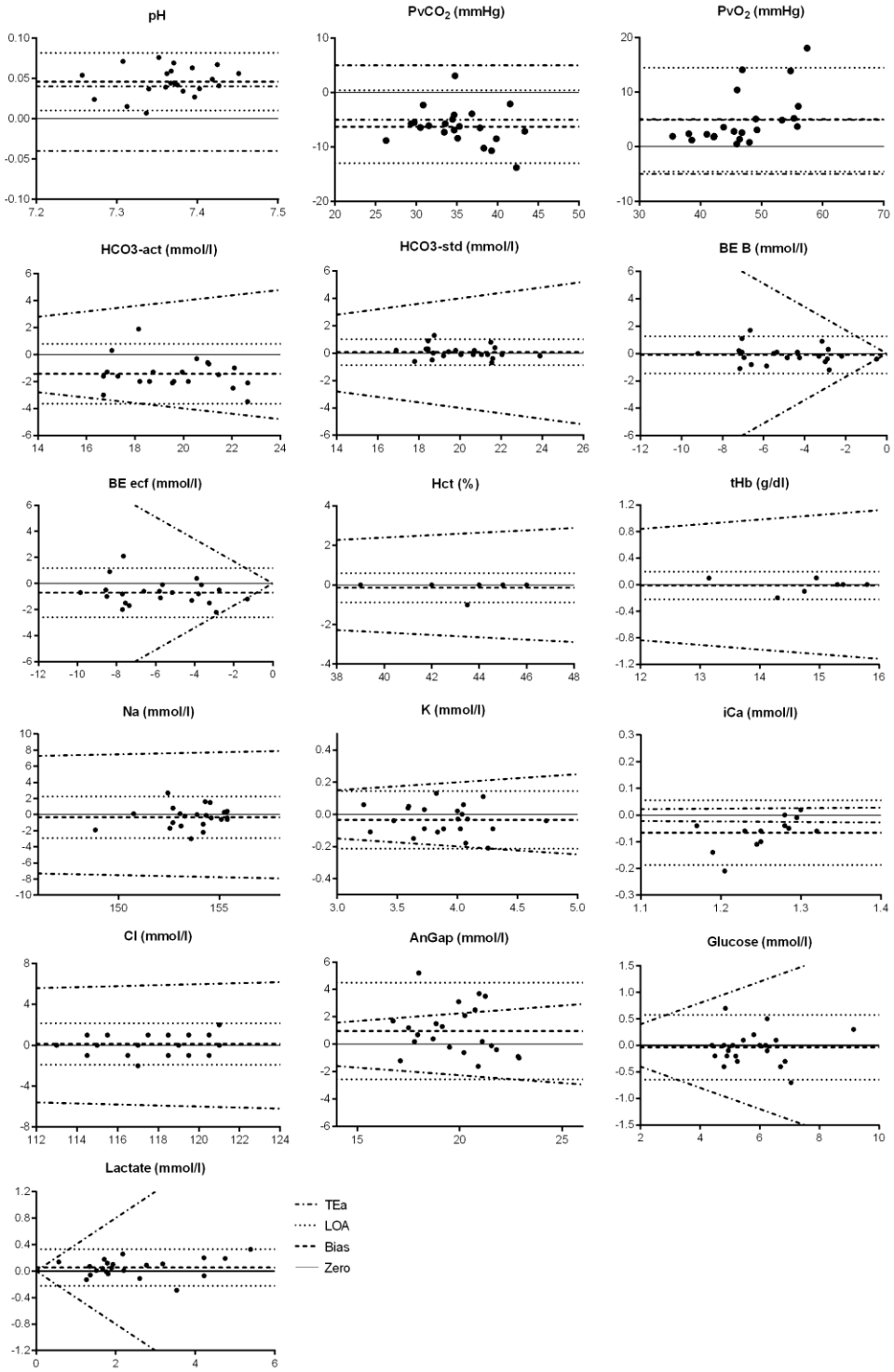

Figure 2: TEa: Allowable total error, LOA: limits of agreement, grey shading indicates values exceeding TEa.

Figure 3: Passing-Bablok regression plots

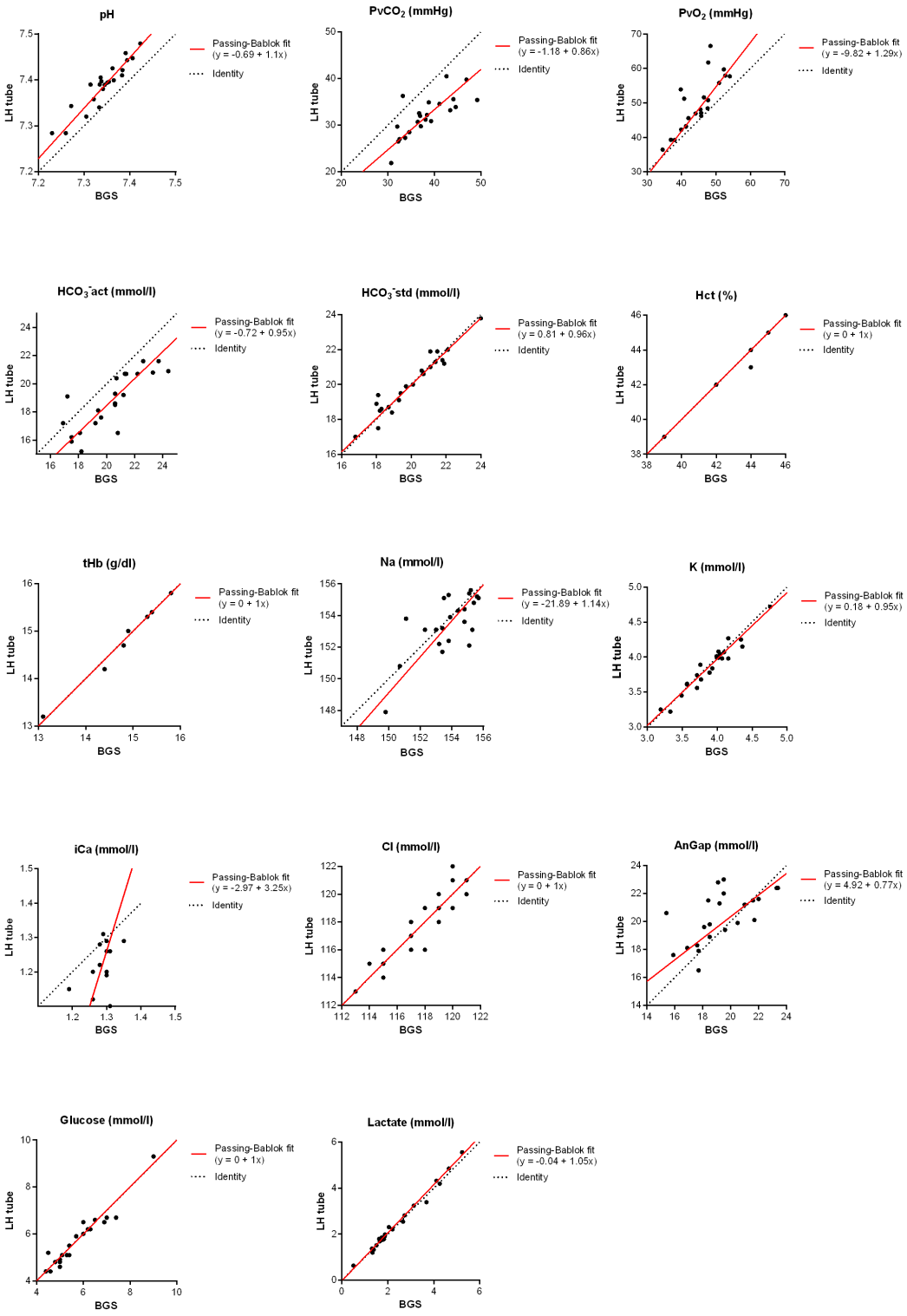

Figure 3: BGS: blood gas syringe, LH tube: multi-purpose lithium heparin tube.

Supplementary Material

Table S1: Haematology and serum biochemistry parameters

Haematology	Serum biochemistry	Coagulation Parameters
• Complete blood count	• Total bilirubin • Glucose • Urea • Creatinine • Total protein • Albumin • Cholesterol • Triglycerides • Alkaline phosphatase • Lipase • Asparagine amino transferase • Alanine amino transferase • Creatinine kinase • Sodium • Potassium • Chloride • Total calcium • Phosphate • Fibrinogen	• Prothrombin time • Partial thromboplastin time • Thrombin time

Table S1: Blood parameters analysed as part of the inclusion criteria

Table S2: Analytical performance RAPIDPoint 500

Parameter	Unit	Concentration	mean	within-run standard deviation	total standard deviation
pH		1	6.718	0.004	0.006
		2	7.103	0.003	0.005
		3	7.324	0.003	0.004
		4	7.524	0.002	0.005
		5	7.700	0.003	0.006
$PvCO_2$	mmHg	1	162.2	4.03	6.53
		2	79.6	2.15	4.23
		3	44.1	1.03	1.88
		4	24.2	0.59	1.00
		5	15.9	0.36	0.92
PvO_2	mmHg	1	46.0	1.80	3.04
		2	74.9	1.14	2.68
		3	105.9	1.32	2.68
		4	142.7	2.42	2.30
		5	501.8	6.82	13.68
tHb	g/l	1	21.0	0.10	0.14
		2	17.4	0.10	0.13
		3	13.6	0.07	0.10
		4	7.6	0.05	0.06
		5	5.0	0.04	0.05
Na	mmol/l	1	112.1	0.68	1.13
		2	112.7	0.34	0.93
		3	132.7	0.53	0.97
		4	153.0	0.61	0.95

		5	169.6	0.61	1.31
K	mmol/l	1	1.43	0.02	0.027
		2	3.11	0.01	0.017
		3	4.98	0.02	0.03
		4	6.56	0.03	0.047
		5	11.49	0.07	0.108
iCa	mmol/l	1	3.05	0.035	0.055
		2	1.53	0.012	0.018
		3	1.19	0.008	0.011
		4	0.77	0.007	0.011
		5	0.55	0.010	0.015
Cl	mmol/l	1	72	0.60	0.730
		2	75	0.64	0.740
		3	98	0.38	0.840
		4	118	0.47	1.250
		5	127	0.67	1.740
Glucose	mg/dl	1	671	10.26	11.8
		2	199	2.85	3.21
		3	94	1.61	1.90
		4	46	1.00	1.34
		5	28	0.80	1.31
Lactate	mmol/l	1	12.15	0.26	0.40
		2	2.90	0.09	0.10
		3	0.88	0.02	0.03

Table S2: Analytical performance of the RAPIDPoint 500 blood gas analyser as stated by the manufacturer (Siemens).